United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Development of Red Blood Cell Protocols for Air Pollution Studies in Rats
LAPR Number:	20-05-001
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6/RTP/USEPA/US
Date Originated:	05/04/2017
LAPR Expiration Date:	05/31/2020
Agenda Date:	05/17/2017
Date Approved:	5/26/2017
Date Closed:	

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	05/26/2017	DMR	
	Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US by Exemption 6 RTP/USEPA/US	05/26/2017	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
Development of Red Blood Cell Protocols for Air Pollution Studies in Rats

Is this a continuing study with a previously approved LAPR?
No

2. Programatic Information

a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.
This research is part of the US EPA's Air Climate and Energy (ACE) Research Action Plan (PEP Tasks 1.1, 1.2, and 1.3).

b. What is the Quality Assurance Project Plan (QAPP) covering this project?
QAPP # E-EPHD-0031079-QP-1-0 (in progress)

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6 Exemption 6 Exemption 6 RTP/USEPA/US	EPHD *Branch* CIB	MD

4. Alternate Contact:

Alternate Contact	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US	EPHD *Branch* CIB	MD

SECTION A - Description of Project

1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the

continuation. Please spell out all acronyms and abbreviations with their initial use.

The primary objective of this project is to develop laboratory techniques that can evaluate the role of red blood cells (RBCs) in air pollution-induced cardiovascular dysfunction. This is a Pathfinder Innovation Project (PIP) 6 Stage 1 awarded project. RBCs are the most abundant cell type in the body, are paramount to survival (i.e. oxygen transport), and with 100% of RBCs passing through the lungs, the site of air pollution exposure, may serve as dynamic sensors and first-responders to environmental stress. Studies have shown that RBCs play a role in cardiovascular disease and are capable of regulating heart, blood vessel, and immune function. However, RBCs are routinely discarded in air pollution studies in favor of targets of much lower abundance based on the misunderstanding that RBCs are only benign oxygen delivery agents. This project will evaluate RBC membrane deformability, oxidative stress, and signaling molecule release for suitability for rapid screening and use as biological initiating events/biomarkers of cardiovascular responses to environmental stress.

This project has 2 objectives. The first is to develop and optimize several assays to be performed after RBCs are collected and isolated. Thus a cohort of blood donor rats will be used for this objective. The three assays being developed at this time are a) RBC Oxidation Capacity Assay, b) RBC ATP Flash Assay, and c) RBC Shape and Deformation Assay. Secondly, after assay development and optimization, a particulate matter (PM) exposure study will be conducted in order to examine the impacts of exposure on RBC parameters.

Ultimately, this research effort supports the explicitly stated needs of the United States Environmental Protection Agency's (US EPA) Air Climate and Energy (ACE) Research Action Plan (PEP Tasks 1.1 and 1.3, and CSS, Topic 3, by 1) enabling rapid screening of biological responses to air pollution and other environmental stressors; 2) accelerating acquisition and processing of toxicity information; 3) expediting identification of most of toxic sources; and 4) opening an avenue of minimally invasive human/ecosystem health and epidemiological screening efforts.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

Dynamic interplay between the lungs, circulating cells, the heart and blood vessels, immune system, and endocrine organs is complex, especially during air pollution exposure. In order to identify the role of red blood cells during this complex process it will be essential to conduct these studies in animals. To date, the use of animals is the only means available to study a level of physiological complexity comparable to that of humans and thus better predict the impact of PM exposure on human health.

b. Justify the species requested:

The rat is the species of choice for our studies because:
1) Rats have the size necessary to allow for ample blood volume collection so that during RBC assay development fewer numbers of rats may be needed compared to smaller rodents like mice.
3) Rat models are well described and utilized frequently in cardiovascular toxicity and pathology studies. This will allow us to examine how our findings relate to other pertinent data in the greater scientific community.

3. How was it determined that this study is not unnecessary duplication?

Pubmed searches for "particulate matter inhalation and erythrocyte", "particulate matter exposure and erythrocyte", "red blood cell and air pollution", and "erythrocyte and air pollution" all yield similar results. While hematological results are reported no studies have examined changes in the functionality of RBCs following air pollution exposure, especially not with the key functional endpoints we wish to study. Thus, there is no evidence in published literature that suggests this study will be a reproduction of any previously published work.

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

This project will utilize 10-12 week old male Sprague-Dawley rats with body mass ranging from 220-320 grams. The study will utilize a cohort of rats for procedure development, another cohort for PM aspiration studies, and an additional cohort for cardiac ultrasound.

In rats used for assay development, blood will be collected using a terminal, large volume blood draw procedure during euthanasia. We will administer 200 mg/kg pentobarbital/phenytoin i.p to each rat while awake and upon reaching plane of deep general anesthesia, assessed by lack of response to multiple firm toe pinches, a gross laparotomy will be performed and 3-9 mL of blood will be drawn from the abdominal aorta using BD vacutainer needles and tubes, followed immediately by exsanguination.

After assay development and optimization is completed, then a standard biomass PM extract toxicity study will be performed to test the impacts of exposure on RBC parameters. This study will utilize oropharyngeal aspiration of PM suspensions (no more than 0.5 mg per rat in 200 µL saline vehicle) or the saline vehicle alone (200 µL), followed by the terminal large volume blood draw procedure during euthanasia at 2 different time points (1 hour and 24 hours post exposure). Separate groups of rats will be exposed to allow for the early time point blood collection (approximately 1 hour following exposure) and a late time point blood collection (approximately 24 hours after exposure). All rats used for RBC assays will undergo terminal bleeds at the specified time points during euthanasia procedure. At the time of euthanasia (1 or 24 hours post exposure), we will administer 200 mg/kg Pentobarbital/phenytoin i.p to each rat while awake and upon reaching plane of deep general anesthesia, assessed by lack of response to multiple firm toe pinches, a gross laparotomy will be performed and 3-9 mL of blood will be drawn from the abdominal aorta using BD vacutainer needles and tubes, followed immediately by exsanguination.

A separate cohort of rats will be used for cardiac ultrasound, which will be performed under isoflurane anesthesia, 24 hours after PM (or vehicle control) exposure. These rats will be injected i.p. with 200 mg/kg pentobarbital/phenytoin while under isoflurane anesthesia. Upon reaching plane of deep general anesthesia, assessed by lack of response to multiple firm toe pinches, a gross laparotomy will be performed, followed by exsanguination.

Non-invasive, whole body plethysmography will be performed immediately following PM and vehicle control exposure on all rats exposed to PM or vehicle control suspensions, and again on all rats remaining 24 hours following exposure to PM and vehicle control (including those used for ultrasound) and be no more than 1 hour in duration at each time point.

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

The overall aim of this project is to develop assays for blood samples and, as such, we cannot run a true sample size analysis. Based on our previous work with air pollution studies and heart/blood vessel endpoints n = 8 is usually calculated with sample size analysis.

The following is a description of exposure groups and animal numbers:

Assay development:

· Naïve untreated rats: N = 50 (Note: we will use the minimum number of rats necessary for optimization)

PM aspiration study

· Saline aspiration/1 hour time point: N = 8
· Saline aspiration/24 hour time point: N = 8
· 50 µg aspiration/1 hour time point: N = 8
· 50 µg aspiration/24 hour time point: N = 8
· 0.5 mg aspiration/1 hour time point: N = 8
· 0.5 mg aspiration/24 hour time point: N = 8

Cardiac ultrasound group: PM oropharyngeal aspiration

- Saline aspiration/cardiac ultrasound: N = 8
- 50 µg aspiration/cardiac ultrasound: N = 8
- 0.5 mg aspiration/cardiac ultrasound: N = 8

Total rats = 122 rats

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	122	
D) Potential pain/distress relieved by appropriate measures:		
E) Unrelieved pain/distress:		

4. Does this LAPR include any of the following:

- ☐ Restraint (>15 Minutes)
- ☐ Food and/or water restriction (>6 Hours)
- ☐ Survival surgery
- ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

For oropharyngeal aspirations (OA): Procedure to take place in Exemption 6 Exemption 6. Rats will be induced with 3-4% isoflurane in 1 to 2 L/min O2. While anesthetized, rats will be briefly suspended by incisors, the tongue exteriorized with padded forceps, and up to 200 µL saline solution containing PM extract will be instilled into the oropharynx using a micropipette. The tongue will be exteriorized (to prevent swallowing) until the instilled droplet is completely aspirated. The rat will then be allowed to wake in a whole body plethysmography chamber so that ventilatory function can be assessed immediately following OA. Food and water will be restricted during whole body plethysmography (no more than 1 hr is expected). There will be a minimum of 2 days of acclimation to whole body plethysmography chambers prior to exposure. Chamber temperature, humidity, and air flow will be continuously monitored throughout the assessments. This system is located in Exemption 6.

For ultrasound: Procedure to take place in Exemption 6. One day following instillation, general heart function and blood flow parameters will be evaluated with high frequency ultrasound using a VisualSonics Vevo 2100 in room Exemption 6 Exemption 6 Exemption 6. Rats will be placed in a warmed induction chamber (plexiglas chamber wrapped with a heated warm water blanket) and anesthetized with 3-4% Isoflurane delivered in medical grade O2 at 1-2 L/min. Rats will then be moved to a heated procedure table where anesthesia will be maintained with 1-3% Isoflurane with medical grade O2 at 1-2 L/min via a nose cone. The eyes will be coated with artificial tears eye ointment to prevent ocular drying. The ventral aspect of the thorax and abdomen will be shaved with electric hair clippers and then a depilatory agent (e.g. Nair) will be applied to the shaved area to remove the remaining short fur (hair and fur interfere greatly with ultrasound). The depilatory agent will be carefully wiped away clean with dry gauze pads. With the rat in dorsal recumbency, each paw will be gently taped to ECG electrodes coated with electrode cream and a rectal probe will be inserted to monitor body temperature. Prewarmed ultrasound gel will be applied to the chest and abdomen and heart and blood vessel function will be assessed. Standard echocardiographic measurements will be collected: parasternal long axis view of the heart (M-mode data), short axis view (strain-strain rate analyses), and apical view (Doppler of transmitral blood flow), abdominal vascular bed blood flows (e.g. superior mesenteric artery, renal artery, etc.). Rats will be monitored at all times while under anesthesia. The ultrasound procedure, start to finish, will last approximately 10-15 minutes. At the end of the procedure, the rat will be immediately euthanized via i.p. injection of 200 mg/kg pentobarbital/phenytoin (diluted 1/2 with pharmaceutical grade saline). Upon reaching plane of deep general anesthesia, assessed by lack of response to multiple firm toe pinches, a gross laparotomy will be performed, followed by exsanguination as secondary confirmation of death.

For terminal bleeds: Procedure to take place in Exemption 6. At one hour or one day following exposure to test substances (2 separate cohorts, see section B2), rats will be administered 200 mg/kg pentobarbital/phenytoin with an i.p. injection while awake. After injection of pentobarbital/phenytoin, the rat will be checked for non-responsiveness, by multiple firm toe pinches. Arterial blood will be collected by performing a gross laparotomy to expose the abdominal aorta with 3-9 mL of blood drawn from the abdominal aorta using vacutainer needles and tubes. Secondary confirmation of death will be exsanguination.

b. Survival Blood Collections (method, volume, frequency):

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:
Monitoring will be performed by Exemption 6Exemption 6Exemption 6 during the initial anesthesia induction, during OA, during anesthesia recovery, and following all blood draws until normal grooming patterns resume. All animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily before and after each procedure. Animals will be weighed every 3 days and tracked for sudden weight loss (>10%).

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):

c. Testing methods:

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of

sutures/staples, frequency and duration of monitoring including weekend and holiday care):

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?

◯ Yes ◯ No

f. Identify any surgical procedures performed at other institutions or by vendors:

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.

All animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily. If signs of distress or other deleterious effects are observed, all animals from the treatment group will be isolated in a clean control atmosphere and observed for recovery trends. They may be reused for the study if recovery is demonstrated; otherwise, they will be euthanized. The attending veterinarian may be consulted to determine the appropriate course of action.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.

One or more of the following: weight loss (> 10%), labored breathing, abnormal gait, loss of appetite, or unexplained lesions.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study: 122

b. Animals to be transferred from another LAPR:

LAPR Number that is the source of this transfer:

c. Animals to be transferred from another source:

d. Offspring produced onsite (used for data collection and/or weaned):

e. TOTAL NUMBER of animals for duration of the LAPR 122

2. Species (limited to one per LAPR): Rat(s)

3. Strain: Sprague Dawley rat(s)

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

na

4. Sources of animals:

Charles River

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6 (Animal Care Facility)

- Initial Housing

Exemption 6
Exemption 6

- Oropharyngeal aspiration and whole body plethysmography

Exemption 6

Ultrasounds, bleeds, and necropsies

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

No *Room Numbers:*

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

na

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

na

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

na

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

Envirodry will be provided as enrichment in each cage with pine shavings bedding and rats will be housed 2 per cage.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

Particulate Matter: one of the following: wildfire/biomass burn PM, residual oil fly ash PM, diesel exhaust PM. No identified LD50. Will be instilled once via oropharyngeal aspiration at a maximum dose of 0.5 mg/rat.

Euthanasia agent:
Sodium pentobarbital/phenytoin (Pharmaceutical grade). Maximum dose of sodium pentobarbital to be administered = 200 mg/kg i.p., Maximum dose of Phenytoin to be administered = 25 mg/kg i.p., Pentobarbital LD50 rat, i.p. = 108 mg/kg, mouse i.p. = 123 mg.kg. Phenytoin LD50 rat i.v. = 101 mg/kg, mouse i.p. = 100 mg/kg. PPE (safety glasses, gloves, lab coat) will be worn by all personnel at all times during its use.

Anesthetic:
Isoflurane (Pharmaceutical grade): Maximum concentration is 3-4% for induction and 1-3% for maintenance. LC50 rat inhalation = 15,300 ppm for 3 hours, mouse inhalation = 16,800 ppm/3 hr. Isoflurane is considered a "potentially hazardous substance" but does not require an HSRP. Isoflurane will be used in the fume hood in A552 or A463-A, or at the down draft sinks in A584 or A587 and delivered with 1.0-2.0 L/min O2, and standard PPE (safety glasses, gloves, lab coat) will be worn by all personnel at all times during its use.

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.

Drugs will be pharmaceutical grade. PM, a combustion byproduct, is not available in pharmaceutical grade.

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	study coordination; animal handling/care; necropsies;	19 years of experience in use of laboratory animals; >10 years inphysiological monitoring; completed NHEERL required

		physiological monitoring	training
Exemption 6	Post-Doc	study coordination; animal handling/care; necropsies; physiological monitoring	8 years of experience in use of laboratory animals, and physiological monitoring; completed NHEERL required training
Exemption 6	Associate Principal Investigator	study coordination; animal handling/care; necropsies; physiological monitoring	18 years of experience in use of laboratory animals; >10 years inphysiological monitoring; completed NHEERL required training
Exemption 6	Student	animal handling/care; necropsies, physiological monitoring	>1 year of experience in use of laboratory animals and physiological monitoring; completed NHEERL required training
Exemption 6	Technical Staff	animal handling/care; necropsies, physiological monitoring	>26 years of experience in use of laboratory animals and and physiological monitoring; completed NHEERL required training
Exemption 6	Student	animal handling/care; necropsies, physiological monitoring	>2 years of experience in use of laboratory animals and physiological monitoring; completed NHEERL required training
Exemption 6	Technical Staff	animal handling/care; physiological monitoring	>25 years of experience in use of laboratory animals and and physiological monitoring; completed NHEERL required training
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

All animals used for assay development will be euthanized at the time of the terminal bleed procedure. All animals slated for the PM studies will be euthanized following completion of experimental procedures no more than one day after oropharyngeal aspiration.

2. Describe the euthanasia techniques:

Method(s): Euthanasia plus exsanguination

Agent(s): Sodium pentobarbital/phenytoin
Dose (mg/kg): Overdose of Sodium pentobarbital/phenytoin (200 mg/kg; 25 mg/kg)
Volume: approximately 0.25 ml/rat for a 250 g rat (200 mg/ml solution)
Route: Intraperitoneal

Source(s) of information used to select the above agents/methods:
_ Veterinary Staff, 2013 AVMA Guidelines on Euthanasia.

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).
na

4. Describe how death is to be confirmed.
exsanguination

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.
Euthanized as above
Euthanized by Animal Care Contractor
Transferred to another study
The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?
● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date

Exemption 6 Exemption 6	05/04/2017

Submitted: 05/04/2017

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	***Approval Date***	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	05/04/2017 by Exemption 6 Exemption 6 RTP/USEPA/US	Exemption 6 ***Lotus Notes Address*** Exemption 6 Exemption 6 /RTP/USEPA/US	EPHD ***Branch*** CIB	MD ***Submitted to Branch Chief for Approval*** 05/04/2017 11:18 AM

ATTACHMENTS

20-05-001 PI resp1.pdf

Actions

First Update notification sent: 04/03/2018
Second Update notification sent:
First 2nd Annual notification sent:

Second 2nd Annual notification sent:

1st Expiration notification sent:
2nd Expiration notification sent:

History Log: